

2018-12

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<http://hdl.handle.net/10026.1/12350>

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10.1016/j.jenvrad.2018.07.005

Journal of Environmental Radioactivity

Elsevier

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**Relative comparison of tissue specific bioaccumulation and radiation dose estimation in marine and freshwater bivalve molluscs following exposure to phosphorus-32**

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## Highlights

- $^{32}\text{P}$  accumulation and depuration studied in two bivalve species
- $^{32}\text{P}$  showed a concentration dependant uptake in bivalves
- Tissue specificity evident, independent of species
- Digestive gland showed the highest  $^{32}\text{P}$  bioaccumulation
- Tissue specific dose rates in some cases significantly higher than whole-body averages

## Abstract

With respect to environmental protection, understanding radionuclide bioconcentration is necessary to relate exposure to radiation dose and hence to biological responses. Few studies are available on tissue specific accumulation of short-lived radionuclides in aquatic invertebrates. Short-lived radionuclides such as  $^{32}\text{P}$  (Phosphorus ( $^{32}\text{P}$ )), although occurring in small quantities in the environment, are capable of concentrating in the biota, especially if they are chronically exposed. In this study, we firstly compared tissue specific bioaccumulation and release (depuration) of  $^{32}\text{P}$  in adult marine (*Mytilus galloprovincialis*, MG) and freshwater bivalve molluscs (*Dreissena polymorpha*, DP). Secondly, using the Environmental Risk from Ionising Contaminants Assessment and Management (ERICA) tool, we calculated tissue specific doses following determination of radionuclide concentration. Marine and freshwater bivalves, were exposed for 10 days to varying  $^{32}\text{P}$  concentrations to acquire desired whole body average dose rates of 0.10, 1.0 and 10 mGy d<sup>-1</sup>. Dose rates encompass a screening dose rate value of 10  $\mu\text{Gy h}^{-1}$  (0.24 mGy d<sup>-1</sup>), in accordance with the ERICA tool. This study is the first to relate tissue specific uptake and release (*via* excretion) of  $^{32}\text{P}$  from two anatomically similar bivalve species. Results showed highly tissue specific accumulation of this radionuclide and similarity of accumulation pattern between the two species. Our data, which highlights preferential  $^{32}\text{P}$  accumulation in specific tissues such as digestive gland, demonstrates that in some cases, tissue-specific dose rates may be required to fully evaluate the potential effects of radiation exposure on non-human biota. Differential sensitivity between biological tissues could result in detrimental biological responses at levels presumed to be acceptable when adopting a 'whole-body' approach.

**Keywords:** Bioaccumulation; Radiation; Bivalves; Uptake,  $^{32}\text{P}$  Phosphorus; ERICA tool

**Abbreviations:** AM, Adductor muscle; Bq, Becquerel; CF, Concentration factor; DG, Digestive gland; DP, *Dreissena polymorpha*; ERICA, Environmental Risk from Ionising Contaminants: Assessment and Management (ERICA); IMW, Internal mussel water; IR, Ionising radiation; LSC, Liquid Scintillation Counting; ME, *Mytilus edulis*; MG, *Mytilus galloprovincialis*; mGy d<sup>-1</sup>, Milligray per day; y, Year

## 1. Introduction

Short lived radionuclides such as  $^{32}\text{P}$  (Phosphorus-32, radiophosphorus), although occurring in small quantities in the environment, may be capable of accumulating in aquatic biota (Smith et al. 2011). This is particularly so if the radionuclide is continuously discharged in the environment, and the biota is chronically exposed. In this context,  $^{32}\text{P}$  is discharged into aquatic systems from various sources. For example, in England and Wales, 7, 5.2 and 5.7 GBq of  $^{32}\text{P}$  was discharged in 2015 as liquid waste from educational, medical (i.e. hospitals) and other establishments (e.g. research, manufacturing and public sector) respectively (RIFE 2015). In terms of environmental concentrations,  $^{32}\text{P}$  reference conditions in Scotland (i.e. concentrations that result in a total ingested dose for humans of  $0.10 \text{ mSv y}^{-1}$  if consumed at  $2 \text{ L day}^{-1}$ ), are set at  $57 \text{ Bq L}^{-1}$  (DWQR 2014), with recorded values (2005-2013) averaging  $0.27 \pm 0.21 \text{ Bq L}^{-1}$  in the River Clyde (Erskine Harbour, King George V Dock), Scotland (SEPA 2013).  $^{32}\text{P}$  was chosen due to ease of use in an experimental setting and as a surrogate for beta/ gamma emitting radionuclides  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$ . Phosphorus in the natural environment serves as an essential nutrient, and in common with non-radioactive counterpart, radioactive phosphorus ( $^{32}\text{P}$ ) would have similar exposure pathways and bioaccumulation pattern in the tissues.

In terms of human health protection, contaminated organisms could pose a risk to health via the food chain (Jha 2004, 2008; Aoun et al. 2015; Yang et al. 2015).  $^{32}\text{P}$  uptake in humans may occur *via* dietary pathways, with dose being higher in the foetus and breastfed infants, than the adult (Oatway et al. 2008). Understanding radionuclide concentration patterns in biota allows for the development of adequate protection strategies, with the aim of reducing potential human dose while maintaining environmental sustainability. Despite continuous and prolonged use in industry, and subsequent discharges, no studies to our knowledge have investigated tissue specific accumulation of  $^{32}\text{P}$  in aquatic biota.

Bioaccumulative abilities in aquatic bivalves, an important group of invertebrates of ecological and economic importance, has been identified in scientific literature. This is notably to ubiquitous, long-lived radionuclides such as  $^{134}\text{Cs}$ ,  $^{210}\text{Po}$ ,  $^{210}\text{Pb}$  and  $^3\text{H}$  (Evans 1984; Jha et al. 2005; Kalaycı et al. 2013; Feroz Khan et al. 2014; Dallas et al. 2016a; Metian et al. 2016; Pearson et al. 2018). However whole body accumulation and dose are often (but not always) the focus of such studies. Sufficient data are not available for tissue specific accumulation of short-lived radionuclides. It is well accepted that in common with other contaminants (Al-Subiai et al., 2011, 2012; Dallas et al., 2013; Di et al., 2011, 2017), radionuclides accumulate in the biota in a tissue specific manner. Whole-body determination of radionuclide bioaccumulation levels is important for risk assessments, however for biomonitoring and biological response studies (including sensitive transcriptomics and proteomics studies), it is important that tissue specific information is generated. Radionuclide uptake disparity amongst tissues has been highlighted in studies from Jha et al (2005), Jaeschke et al (2011), Dallas et al (2016a) and Pearson et al (2018) where tritium accumulation in bivalve (*Mytilus sp.*) tissues were observed to be highly specific. Digestive gland (hepatopancreas/gut), gill and foot showed higher concentrations following exposure to varying amounts of tritium (5-15 MBq L<sup>-1</sup>). Such trends are followed in green and brown mussels (*P. perna*, *P. indica*), where digestive gland showed maximum  $^{210}\text{Po}/^{210}\text{Pb}$  activity over other biological soft tissue and shell (Feroz Khan and Godwin Wesley 2012). Furthermore, in scallop (*Pecten maximus*) soft tissue,  $^{241}\text{Am}$  was predominantly concentrated in the mantle and digestive gland, whereas  $^{134}\text{Cs}$  was mainly present in the adductor muscle and mantle (Metian et al. 2011). In environmental protection terms, understanding radionuclide accumulation is necessary to relate exposure, to radiation dose and to determine potential biological responses. Exposure to ionising radiations (IR) can occur via multiple aqueous and dietary pathways, the behaviour and fate of radionuclides when accumulated in specific biological tissues or organs in the aquatic biota could be influenced by many factors and may vary significantly under different exposure scenarios (Pearson et al., 2018). Given that radionuclides accumulate differentially in the tissues, from a biomonitoring perspective, whole-body bioaccumulation monitoring is therefore not necessarily sufficient in fully protecting aquatic biota from the exposure. This is particularly important as differential tissue sensitivity could result in a detrimental biological response at levels presumed to be acceptable.

Dosimetry models, such as the Environmental Risk from Ionising Contaminants Assessment and Management (ERICA) Tool have been developed to evaluate radiological risk to aquatic and terrestrial biota (Brown et al. 2008). Risk is assessed by comparing a dose rate in a reference organism to a dose rate of  $10 \mu\text{Gy h}^{-1}$  ( $0.24 \text{ mGy d}^{-1}$ ), a “screening dose rate” whereby no effect to populations of biota is expected (Garnier-Laplace and Gilbin 2006; Garnier-Laplace et al. 2008). Though dosimetry models are of great assistance in radiobiological research, ERICA tool predicted dose rates presume homogeneous radionuclide distribution within biota, which are represented as ellipsoidal shapes (Beresford et al. 2007). In order to adequately estimate radiological risk to biota, we require a greater knowledge of tissue specific radionuclide concentrations in a range of organisms, the transfer pathways, concentration factor, dose rate and an evaluation of any possible biological effects are required. Such data may also help pinpoint key tissues of interest for biomonitoring purposes.

The presence of radionuclides is of concern for both marine and freshwater environments. The marine species *Mytilus galloprovincialis* (MG) and freshwater *Dreissena polymorpha* (DP) were therefore selected in this study (Figure 1). Although marine species might not be used to assess the risk in the freshwater environment or vice-versa, it is nevertheless important to estimate relative radionuclide accumulation in the biota belonging to same biological or taxonomic group. This would help to identify the most sensitive species for environmental protection. These two species exhibit anatomical similarities, prevalence within respective water bodies and have known ability to concentrate contaminants within tissues. They are widely distributed and extensively used for ecotoxicological studies (Bayne 1976; Chatel et al. 2012; Dallas et al. 2012, 2013; Binelli et al. 2015; Jaeschke et al. 2015; Banni et al. 2017). They serve as important monitoring systems in coastal and inland water systems (Viarengo et al. 2007; Bourgeault et al. 2010; Binelli et al. 2015; Sforzini et al. 2018), and are important components in marine and freshwater food chains (Bayne 1976; Prejs et al. 1990). In addition, marine bivalves such as *Mytilus* sp. are considered an important protein source to humans. Consumption of contaminated mussels may result in higher human radiation doses (Macklin Rani et al. 2014; Cho et al. 2016), from isotopes such as  $^{210}\text{Pb}$ ,  $^{210}\text{Po}$ ,  $^{40}\text{K}$  and  $^{137}\text{Cs}$  (Alonso-Hernandez et al. 2002; Assunta Meli et al. 2008; Kılıç et al. 2014).

The present study had the following aims and objectives: (a) to determine tissue specific accumulation and depuration (release via excretion) of  $^{32}\text{P}$  in two different species of mussels (i.e. marine and freshwater) (b) to evaluate the application of the ERICA tool in determining tissue specific radiation doses and (c) to identify the accumulation pattern of  $^{32}\text{P}$ , as to highlight key tissues of interest for future experiments investigating potential biological responses. It was hypothesised that whole body concentration of  $^{32}\text{P}$  would be comparable in freshwater and marine bivalves, and that accumulation would be tissue specific.

## **2. Materials and methods**

### **2.1. Chemicals and suppliers**

Commercially available, radiolabelled-ATP (Adenosine triphosphate,  $\gamma$ - $^{32}\text{P}$ ) was obtained from Perkin Elmer (PerkinElmer, UK) in batches of 9.25 MBq (specific activity: 370 MBq mL<sup>-1</sup>) and used as the source of radioactive  $^{32}\text{P}$  for our experimental purposes. Radiolabelled ATP was utilised in our experiments as (a) due to its readily, bioavailable form would be accumulated readily into tissues, (b) the ATP itself would not cause biological damage as the radioisotope is almost chemically identical to the stable isotope, it therefore would not affect future experiments and (c) it would not affect the chemical composition (i.e. pH, salinity) of the sea/freshwater. Radiolabelled-ATP was diluted with DI water to form appropriate working solutions. Working solution added to beakers was decay adjusted. Nitric acid was obtained from Fisher Scientific UK (Nitric acid 68%, Primar Plus™) and scintillation cocktail from LabLogic systems Ltd. UK (ScintLogic, UK). All other chemicals and reagents were purchased from Anachem Ltd. UK, Sigma-Aldrich Company Ltd UK, VWR International Ltd USA or Greiner Bio-One Ltd UK, unless stated otherwise. Additional product details are mentioned in text as appropriate.

### **2.2. Mussel exposure conditions**



Two ten-day exposures were performed between December-February 2016-17. Adult MG and DP were collected from Trebarwith strand (Dallas et al., 2013) and Bude, Cornwall, UK (50.828059, -4.549053), respectively. Maintenance of the mussels has been described in detail in previous publications (Dallas et al. 2013; Dallas et al. 2016a; Pearson et al. 2018). As Hilbish et al. (2002) reported the occurrence and distribution of *Mytilus edulis*, *M. galloprovincialis* and their hybrids in the coastal regions of south-west England, we ensured species homogeneity in our experiments based on the method of Inoue et al. (1995). This technique, which used polymerase chain reaction (PCR) primers to amplify a specific region of the Glu-5' gene (Gene Bank accession no. D63778), confirmed the species used in our laboratory to be *M. galloprovincialis* (Pearson et al., 2018). We further confirmed the species prior to the present set of experiments using the same methodology (data not included).

MG was maintained in UV treated, filtered ( $< 10 \mu\text{m}$ ), aerated seawater and DP in an artificial river water solution, both species were maintained at a 12:12 photoperiod at  $15^\circ\text{C}$ . Three MG and fourteen DP individuals per beaker (total weight of 35 g/beaker) were exposed to the following activity concentrations of  $^{32}\text{P}$  in triplicate: 709, 7090 or 70900 and 571, 5710 or 57100  $\text{Bq L}^{-1}$ , respectively, along with control treatments. The sample size (number of individuals) used in the study was decided to obtain a statistically robust set of data and was in line with previous studies (Dallas et al. 2016b). Activity concentrations in water were calculated from preliminary experiments (data not included). Water changes (50 %) were carried out on days 3, 5, 7 and 9 and mussels were fed during this exposure (2 hours before each water change), as described in detail elsewhere (Dallas et al. 2016a). MG were fed a solution of *Isochrysis galbana* algae ( $1.05 \times 10^{-5}$  cells  $\text{mL}^{-1}$ , Reed Mariculture, Campbell, CA, USA), DP fed on dried *Chlorella* powder (3.2 mg/mussel per feed, Naturya, Bath, UK). Water samples (1 mL, in duplicate) were taken around 30 minutes after each water change, and processed for liquid scintillation counting (LSC) to determine water activity concentrations.

Water quality parameters were measured routinely, before and after water changes. Parameters were found to be within acceptable range (pH  $8.7 \pm 1.2$ , temperature  $14.5 \pm 1.8^\circ\text{C}$ , dissolved oxygen (DO)  $96.9 \pm 8\%$  and salinity  $36.7 \pm 0.6$  for MG and pH  $8.1 \pm 0.3$ , temperature  $14.8 \pm 0.9^\circ\text{C}$ , DO  $92.3 \pm 4.1\%$  and salinity  $0.3 \pm 0$  for DP).

### **2.3. Sampling procedures and Liquid scintillation counting**

At the end of the exposure period, water from beakers was drained through a sieve (Fisherbrand, ISO 3310/1 250 µM). Faeces and pseudo faeces were collected from sieve on a weighed section of tissue, and placed into pre-weighed tubes (Punt et al. 1998; Jha et al. 2005). Samples were freeze dried (< 12 h, or until pressure is constant at 50-60 µbar), re-weighed and rehydrated (1 mL, DI water). Mussels were dissected and separated into soft tissue (i.e. gill, mantle, adductor muscle, digestive gland and 'other' tissue), shell and internal mussel water (IMW). IMW refers to all water within mantle cavity. To collect IMW, the shell was opened with scissors, and the individual rested over a tube as to drain internal fluid. Samples were re-weighed to get mL/individual. Soft tissues were dissected and placed into pre-weighed tubes, re-weighed and then homogenised in DI water (10 mL). Shells were placed into pre-weighed tubes and re-weighed, then rinsed thoroughly, scrubbed using a sponge and crushed using a hammer and/or pestle and mortar. Shells were solubilised in concentrated nitric acid (5 mL, < 5 hr) at room temperature with occasional shaking (200 rpm), and then diluted in DI water (15 mL).

Soft tissue, shell, IMW or faeces solution (1 mL, in duplicate) were mixed with 4 mL scintillation cocktail (ScintLogic U) in sealed scintillation vials (Fisherbrand™ Borosilicate Glass). 4 mL cocktail was also added to water samples. Samples were left in dark for ~ 2 hours prior to counting (Hidex 300SL), samples were read (10 seconds) in triplicate. Activity concentrations were background corrected by blank subtracting from each sample, the blank was non-spiked fresh or seawater. In accordance with Jaeschke and Bradshaw (2013), CPM values that fell below the blank were assigned an activity of 0.000. All samples were decay corrected.

### **2.4. Dosimetry and the ERICA TOOL**

The Tier 2 assessment module of the ERICA tool was used for dose estimation. <sup>32</sup>P was chosen as one of the ERICA tool's default isotopes (Brown et al. 2008). Custom MG geometry parameters were adopted from Dallas et al. (2016b), for DP, custom parameters were used for accurate dosimetry (data not included). Tissue specific dose rate (e.g. <sup>32</sup>P dose to digestive gland) was determined by

taking mean measurements during sampling (i.e. mass, height, width, length), and developing custom geometry parameters on the ERICA tool (Table 1). A radiation-weighting factor of 1 (ERICA tool's default for high energy beta emitters) was used. The sediment-water distribution co-efficient ( $K_d$ ) was set to  $0 \text{ L kg}^{-1}$ , as no sediment was present in the experimental design. Concentration ratio was set to 0 as actual measured tissue activity concentrations were used. Variable inputs required to calculate total dose rate per organism ( $\mu\text{Gy h}^{-1}$ ) were activity concentration in water ( $\text{Bq L}^{-1}$ ), activity concentration in sediment ( $\text{Bq kg}^{-1}$ ), this is set to 0, and the activity concentration in organism ( $\text{Bq kg}^{-1}$ ). For the latter, total activity (Bq) per beaker was divided by total mussel weight (g, including shell) per beaker, and then multiplied by 1000 to acquire  $\text{Bq kg}^{-1}$ .

## **2.5. Statistical analysis**

All values are mean  $\pm$  SE unless otherwise stated. Statistical analyses were performed in R (1.0.136; [www.r-project.org](http://www.r-project.org)). Data was checked for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test), the non-parametric Kruskal-Wallis test was used to evaluate effects of treatment on bioconcentration. Comparison between treatment groups was determined using a pairwise Wilcoxon test with Holm-Bonferroni correction. Level of significance for all tests was set at  $p < 0.05$  unless otherwise stated.

## **3. Results**

### **3.1. Activity concentrations in water**

Activity concentrations in water (Table. 2) showed good agreement with nominal values at 535, 6911 and  $70253 \text{ Bq L}^{-1}$  for MG and 492, 4089 and  $45611 \text{ Bq L}^{-1}$  for DP. Control water sample activities were below the LOD.

#### **3.2.1. Activity concentrations in bivalve soft tissue, shell and IMW**

In general, there appears to be a fairly high degree of variability between biological tissues (Fig. 2). Order of  $^{32}\text{P}$  accumulation, in terms of total activity (Bq) per gram of tissue, is illustrated in Table 3. Digestive gland showed the highest degree of accumulation over all treatments but DP control (Table. 3), independent of species. 87% (MG) and 45% (DP) of total activity within soft tissue is located in the digestive gland (10 mGy d<sup>-1</sup> treatment). MG digestive gland showed significantly higher values than DP across all treatments.

#### **3.2.1.1. Soft tissue**

Apart from adductor mussel (AM) values between DP control and 0.1 mGy d<sup>-1</sup> ( $p = 1$ ), bioconcentration increased in a dose dependant manner across all the tissues (Fig. 2), difference between treatments was not always statistically significant. In DP mantle and gill, no significance was noted between the control and 0.1 mGy d<sup>-1</sup> treatment ( $p = 0.27$  and  $0.16$ ), this trend was not evident in MG mantle ( $p < 0.01$ ). Mantle and gill values in 1 and 10 mGy d<sup>-1</sup> treatments showed a greater degree of  $^{32}\text{P}$  activity than in controls and 0.1 mGy d<sup>-1</sup> treatments, independent of species. In all 'other' soft tissue, no variation is evident between species control ( $p = 1$ ), or between the DP 0.1 mGy d<sup>-1</sup> and MG control treatments ( $p = 0.11$ ).

#### **3.2.1.2. Internal mussel water (IMW) and shell**

In all  $^{32}\text{P}$  treatments, the lowest activity concentration was found in the IMW, followed by the shell (Table. 3). The activity concentration in IMW of both species is comparable to the nominal activity in water (Bq mL<sup>-1</sup>). In terms of disparity between species, there is no significant difference in control samples ( $p = 0.96$ ), this is also true between the DP 1 and MG 0.1 mGy d<sup>-1</sup> ( $p = 0.96$ ), and DP 10 and MG 1 mGy d<sup>-1</sup> treatments ( $p = 0.57$ ). There is a clear dose dependant response in the bivalve shell ( $p < 0.01$ ), with the highest 10 mGy d<sup>-1</sup> treatments showing the greatest activity concentration. From the 1 to 10 mGy d<sup>-1</sup> treatment, there is an increase in total

activity of 98% (MG) and 90% (DP). In terms of species comparison, there is no significant variance between shell bioconcentration in control treatments ( $p = 0.1$ ).

#### **3.2.1.3. Tissue specific $^{32}\text{P}$ accumulation**

In terms of species, MG had a significantly higher degree of  $^{32}\text{P}$  accumulation in all individual tissues ( $p < 0.05$ ), for all the treatments. Bioconcentration of  $^{32}\text{P}$  was more varied amongst DP tissue compared to MG. Proportionately (Fig. 3), in the 10 mGy d<sup>-1</sup> treatment accumulation was as followed in MG; digestive gland (87 %) > gill (4.5 %) > other (3.9 %) > mantle (2.3 %) > adductor muscle (1.9 %) > shell (0.3 %) > IMW (0.1 %), and digestive gland (44.6 %) > other (16.2 %) > gill (12.5 %) > mantle (10 %) > adductor muscle (9.7 %) > shell (4.8 %) > IMW (2.1 %) in DP (Table 3).

#### **3.2.2. Faecal matter and pseudofaeces**

$^{32}\text{P}$  release (Fig. 4) was determined by activity concentrations in faeces and pseudo-faeces. Due to the experimental set-up it was not feasible to distinguish between the two. In both species, activity concentrations (Bq g<sup>-1</sup> faeces) rise in a dose dependant manner ( $p < 0.001$ ).  $^{32}\text{P}$  concentration in faeces and pseudo-faeces from the 10 mGy d<sup>-1</sup> treatment, was significantly higher than in all treatments ( $p < 0.001$ ), with DP faeces having the greatest total activity at 625.1 Bq g<sup>-1</sup> compared to 466.1 Bq g<sup>-1</sup>. There is however, no statistical variation ( $p = 0.2$ ). Both species independently displayed significant differences between radioactive treatments, but no variation was seen between species; 0.1 mGy d<sup>-1</sup> ( $p = 0.9$ ), 1 mGy d<sup>-1</sup> ( $p = 0.09$ ) and 10 mGy d<sup>-1</sup> ( $p = 0.2$ ). No variance was observed in control bivalves.

#### **3.3. Dosimetry**

Tissue activity concentrations of  $^{32}\text{P}$  reached  $41 \pm 3\%$  of the value of the surrounding water in MG, as opposed to  $17 \pm 3\%$  by DP. Using tier 2 of the ERICA tool, the average achieved total body dose rates were calculated to be 0.07, 0.68 and 7.25 mGy d<sup>-1</sup> for MG, and

0.02, 0.24 and 2.62 mGy d<sup>-1</sup> for DP, falling short of the expected values of 0.1, 1 and 10 mGy d<sup>-1</sup> (Table 4). Table 4 demonstrates water activity concentrations that give the correct dose (Bq L<sup>-1</sup>), corrected to 35 g whole mussel tissue (Inc. soft tissue, shell and IMW)/beaker. Whole mussel tissues (i.e. soft tissue, shell and IMW), as opposed to just soft tissue, were used in dose rate calculations as to more accurately reflect internal dose rate. This is particularly important for future experiments where biological effects are determined in mussel species.

In consideration to the significant degree of accumulation found in the digestive gland, independent of species, a tissue specific dose rate was calculated using the ERICA tool. Dose was determined by creating two new organisms; *D. polymorpha* (DG) and *M. galloprovincialis* (DG), occupancy factors and tissue specific organism geometry are listed in Table 1. Input parameters were mean measurements taken from experimental samples from bioaccumulation experiments. The average achieved dose rates in digestive gland were calculated to be 20.76, 35.28 and 468 mGy d<sup>-1</sup> for the MG, and 0.07, 1.16 and 9.22 mGy d<sup>-1</sup> for DP (Table 5).

To confirm and validate data analysis using the ERICA tool, tissue specific dosimetry calculations were compared to data showing total activity per gram of tissue (Bq g<sup>-1</sup>). In MG, there was a 41% and 92% increase between the 0.1 and 1, and 1 and 10 mGy d<sup>-1</sup> treatment groups in both activity concentrations in tissue (Bq g<sup>-1</sup>) and dose rate. In DP, there was a 94% and 87% increase between the 0.10 and 1, and 1 and 10 mGy d<sup>-1</sup> treatment groups in both activity concentrations in tissue (Bq g<sup>-1</sup>) and dose rate.

Concentration factor values, calculated by dividing tissue-specific <sup>32</sup>P activity concentrations (Bq kg<sup>-1</sup>, wet weight) by activity concentrations of the spiked water, were as follows; 11.7, 11.2 and 11.9 in MG and 3.6, 4.6 and 5 for DP (in 0.1, 1 and 10 mGy d<sup>-1</sup> treatments).

#### 4. Discussions

From this study, it is evident that  $^{32}\text{P}$  accumulation is highly tissue specific, and variable between bivalve species. It is important to note that in this study,  $^{32}\text{P}$  was introduced in a highly bioavailable form (i.e. radiolabelled ATP), demonstrated bioaccumulation patterns in this study may be reflective of this. MG, which accumulated  $41 \pm 3\%$  of  $^{32}\text{P}$  present in the surrounding media as opposed to  $17 \pm 3\%$  by DP, showed a greater degree of  $^{32}\text{P}$  accumulation across all biological tissues. Despite variance in uptake and accumulation,  $^{32}\text{P}$  excretion was comparable between species. It could be assumed that the measured activity concentration takes into account absorption, metabolism of ATP, subsequent dispersal and partitioning of phosphorus in tissue specific manner at a given sampling time. This phenomenon as a whole could be considered as tissue specific accumulation of radiophosphorus. It is also possible that the tissues could have achieved equilibrium over the exposure period. It would, however, be difficult to predict tissue dose delivered by the available radionuclide concentration in the surrounding media. Furthermore, equilibrium status is often regarded as a flaw in the ERICA tool. In terms of dosimetry, the ERICA tool proved valuable in calculating whole body and tissue specific dose rates. Average achieved dose rates were 0.07, 0.68 and 7.25 mGy d<sup>-1</sup> for MG, and 0.02, 0.24 and 2.62 mGy d<sup>-1</sup> for DP, below expected values of 0.10, 1 and 10 mGy d<sup>-1</sup>. The dose dependant nature of  $^{32}\text{P}$  accumulation gives evidence that both marine and freshwater bivalves are suitable bioindicators of radioactive pollution.

In consideration of species, MG accumulated a higher degree of  $^{32}\text{P}$  in biological tissue across all treatments. Such disparity may be a result of several biotic and abiotic variables, including physiology (filtration rates, population density, metabolism, and reproductive stage), biochemistry and water chemistry (salinity, pH, dissolved oxygen, radionuclide speciation) (Nalepa et al. 1991; Reinfelder et al. 1998; Konovalenko et al. 2016; Pearson et al. 2018). The underlying mechanism which leads to differences between freshwater and marine bivalves is unclear, differential radionuclide accumulation between bivalves is a topic with little attention to date. In terms of stable phosphorus (P), tissue bioconcentration has been found to vary dependant on reproductive processes, high P concentrations are evident during periods of spawning in *Mytilus* sp. and DP. (Kuenzler 1961; Jurkiewicz-Karnkowska 2002). Jurkiewicz-Karnkowska

(2002) noted variability in soft tissue P concentrations between three freshwater bivalves (DP, *Anodonta anatina* and *A. cygnea*) inhabiting the Zegrzynski Reservoir, Poland, suggesting species specificity in terms of stable P accumulation.

Feeding and digestion is often regarded as a predominant route of radionuclide intake (McDonald et al. 1993). The digestive gland in bivalves plays a central role in metabolism. It is important for intracellular digestion, as a storage site for metabolic reserves during periods of stress, and as a site of nutrient distribution to other organs, particularly reproductive tissue (Cartier et al. 2004). Under all treatment groups the greatest  $^{32}\text{P}$  concentration was present in the digestive gland, at 87% in MG and 45% in DP of the total activity within soft tissue ( $10 \text{ mGy d}^{-1}$ ), suggesting a dietary route of exposure. The findings are supported by earlier studies by Jaeschke et al (2011) and Jha et al (2005) who reported preferential tritium accumulation in *Mytilus* sp. digestive gland (tritiated glycine,  $1.48 \text{ MBq L}^{-1}$  and tritiated water, 3.7, 37 and  $147 \text{ MBq L}^{-1}$ ). This trend is continued in *Mytilus* spp. following exposure to  $^{241}\text{Am}$ ,  $^{329}\text{Pu}$ ,  $^{237}\text{Np}$  and  $^{63}\text{Ni}$ , (McDonald et al. 1993; Punt et al. 1998) and in marine amphipods exposed to  $^{32}\text{P}$  (Johannes 2003). Variance between marine and freshwater bivalves may result from differential physiological and genetic characteristics. In terms of physiology, filtration rates have been noted as comparable between species, at  $76.6 \text{ (DP)}$  and  $87.5 \text{ ml mussel}^{-1} \text{ h}^{-1}$  (*Mytilus edulis*), along with valve movement at  $90.1 \text{ (DP)}$  and  $92 \text{ (ME)} \%$  of open valves under ambient conditions (Rajagopal et al. 2003). While neither parameter were measured in this study; it is possible that DP individuals are more inclined to close their valves when exposed to  $^{32}\text{P}$ , as a stress response. This behaviour is documented in biofouling control research, where bivalves close valves during periods of water chlorination as a protective strategy (Rajagopal et al. 2003). Observed differences may also result from variable feeding regimes (i.e. species fed different food types) and/or gut physiology. Factors involved with digestion such as food density or quality, gut passage time, volume or retention rate, enzymatic composition, digestive partitioning and chemistry may effect  $^{32}\text{P}$  assimilation (Wang et al. 1995). Despite the disparity in CF values between MG and DP, the trend between biological tissues is similar, suggesting comparability in  $^{32}\text{P}$  accumulation pathways.



Average achieved dose rates in digestive gland were calculated at 20.76, 35.28 and 468 mGy d<sup>-1</sup> for MG, and 0.07, 1.16 and 9.22 mGy d<sup>-1</sup> for DP. This specific tissue dose is substantially greater than calculated whole body doses, suggesting that whole-body dose monitoring may be insufficient in wholly protecting aquatic organisms from radionuclide exposure. Tissue specificity, in terms of accumulation is well documented for many radionuclides. Strontium-90 for example is a 'bone seeker', due to its biochemically similar behaviour to calcium, following ingestion a large proportion will attach to the surface, or be absorbed into bone (ATSDR 2004). In the context of biomonitoring and adequate environmental protection, an understanding of tissue specific dose rates is of high importance. Calculated whole body dose for MG and DP (0.1 and 1 mGy d<sup>-1</sup> treatments) fell below the predicted 'no effects' screening value of 10 µGy h<sup>-1</sup> (0.24 mGy d<sup>-1</sup>), suggesting a minimal risk to the individual or population. However, in all but the DP 0.1 mGy d<sup>-1</sup> treatment, digestive gland dose was above the screening benchmark. The risk to humans via the food chain is dependent on the consumption pattern and duration of radionuclide exposures. This could be influenced by many physico-chemical and biological factors.

In the 10 mGy d<sup>-1</sup> treatment, gill tissue had 1816% (MG) and 255% (DP) less concentrated <sup>32</sup>P than in the digestive gland (Bq g<sup>-1</sup>). As filter feeding organisms, particulates within the water column are captured within cilia on the gills, particulate matter is then carried via mucous strings to the mouth (Riisg et al. 2011). Gill tissue may therefore act as a major pathway for contaminants to enter other biological tissue. IMW activity concentrations are comparable to the expected activity in water (Bq mL<sup>-1</sup>) in both species, suggesting that bivalves are unable to regulate <sup>32</sup>P uptake via aqueous pathways. Past studies have often highlighted gill as a tissue of key concern due to proximity to the surrounding media, high surface area and water content. The relatively low activity in *Mytilus sp.* gill tissue relative to the digestive gland is a trend found in other studies following exposure to tritium (12 to 485 µGy h<sup>-1</sup>) and nickel (<sup>63</sup>Ni) (Punt et al. 1998; Jha et al. 2005). In terms of subsequent biological response, it is important to note that while <sup>32</sup>P may have accumulated to a lesser degree in some tissues, the beta emission can penetrate approximately 0.76 cm of tissue/water (Terrance 2017). By proximity, higher dose rates may be evident in tissue or cells not directly accumulating <sup>32</sup>P to a high degree. In terms of gill

tissue, while a relatively low contaminant concentration is observed, its large surface area and proximity to surrounding media may result in a higher absorbed dose.

In the natural environment, many factors may influence the filtration rate of bivalves, along with feeding and depuration rate. Changing environmental factors, such as water quality conditions, food availability, reproduction and physiological condition may affect feeding behaviour (Riisg et al. 2011). Laboratory conditions may not accurately reflect feeding, and therefore uptake and depuration patterns of  $^{32}\text{P}$  in bivalves may vary. It is also possible that due to different habitats, certain bivalve species are either more adapted to, or have experienced more disturbances or stresses in the wild, and are therefore more resilient to stresses under laboratory conditions. However relative response to a particular stressor of similar magnitude in two different species, representing different habitats, is difficult to estimate in the natural environment. From an environmental protection perspective, an understanding of radionuclide transfer pathways under environmentally realistic conditions, whether uptake is dietary (ingestion of contaminated food) or through direct transfer from surrounding media is important. One of the limitations of the study is that these laboratory-based experiments were carried out in static exposure conditions, which differs from real environmental situations. A flow-through exposure set-up would have been a more realistic experimental design but due to health, safety, logistics and economic reasons (requiring large amounts of radionuclides), a flow-through experimental design was not feasible. Further studies using a wider range of radionuclides and exposure conditions, which better reflect environmental exposure conditions (e.g. flow through system) would be of great benefit. Knowledge of the behaviour and transfer of radionuclides within aquatic systems allows for an assessment of potential impacts and subsequent management strategies.

Understanding excretion of contaminants is important firstly as a means of determining possible chronic effects of assimilated contaminants, and secondly in respects to human consumption. In terms of public health, depuration is mandatory in bivalves harvested for human consumption as to remove contaminants, predominantly bacteria (Lee et al. 2008). The effectiveness of depuration in removing radionuclides is yet to be fully understood. Suspension feeding bivalves produce faeces and pseudofaeces,

the latter of which refers to particles rejected before entering the gut. Excretion of  $^{32}\text{P}$ , measured in a combination of faeces (from alimentary tract) and pseudofaeces (from mantle cavity), do not appear to be consistent with that observed from uptake. In irradiated treatments (0.10, 1 and 10 mGy d<sup>-1</sup>), 0.31%, 0.15% and 0.08% (MG) and 0.4%, 0.15%, 0.34% (DP) of  $^{32}\text{P}$  from surrounding media was excreted; significantly lower than the 41±3% (MG) and 17±3% (DP) of  $^{32}\text{P}$  accumulated within biological tissue. While our findings suggest a slow depuration rate during IR exposure, results are limited in showing a brief snapshot in time. It would be of interest to monitor uptake and excretion, and therefore depuration rates over both a longer duration, and following the removal of  $^{32}\text{P}$  in water.

The shell surface of aquatic bivalves is known to absorb dissolved contaminants from surrounding media (Zuykov et al. 2012), thus why in this study whole body dose was not limited to just soft tissue. When removing both IMW and shell concentrations from the data before ERICA tool analysis, the results follow exactly the same pattern due to the influence of vast  $^{32}\text{P}$  concentrations in the digestive gland.  $^{32}\text{P}$  biosorption in whole shell was concentration dependant in both species, with an increase in total activity of 98% (MG) and 90% (DP) between 1 and 10 mGy d<sup>-1</sup> treatments. Proportionately DP showed higher incorporation into shell, over all treatments, whereas per gram of shell, MG has significantly greater  $^{32}\text{P}$  present. Mollusc shell is formed of a few calcified layers and the periostracum, one thin, organic coating layer (Marin et al. 2012; Zuykov et al. 2012). Species variation may be a result of differing shell microstructure and topography, chemical and macromolecule composition (Marin et al. 2012). As noted by (Zuykov et al. 2012), MG and DP do show disparity in shell topography, where DP has a thinner periostracum and a lamellate surface (Immel et al. 2016). In this study, the content of  $^{32}\text{P}$  in shell was far lower than in soft tissue, this data contrasts to findings by Koide et al. (1982), Metian et al (2011) and Clifton et al (1989) following exposure to radionuclides or heavy metals. As an example, in scallop (*Pecten maximus*), biosorption of  $^{241}\text{Am}$  into the shell was far greater than soft tissue, however in the same species,  $^{134}\text{Cs}$  showed preferential accumulation in soft tissue over shell (Metian et al. 2011). Bivalve shells are widely used to monitor pollutants in the aquatic environment (Zuykov et al. 2013). It is relevant to note that bioconcentration values taken from shell are not reflective of soft tissue values.

## 5. Conclusions

With respect to species comparison between marine and freshwater bivalves, there is limited information available in the scientific literature. This is the first study to compare uptake and depuration (via excretion) of short-lived radionuclide,  $^{32}\text{P}$  in two anatomically similar bivalve species.  $^{32}\text{P}$  accumulation is highly tissue specific, with the majority located within the digestive gland. This is particularly important in the context of biomonitoring and adequate environmental protection, where whole-body dose monitoring may not always be sufficient to protect aquatic organisms from radionuclide exposure. Differential sensitivity between biological tissues could result in harmful biological response at activity levels presumed to be safe. The next step is to link radioactive exposure, accumulation and dose rate, to consequent biological responses. Accumulation within mussel tissues, even for short durations may potentially have long lasting effects in both exposed individuals and subsequent generations. Lastly, considering species variation in  $^{32}\text{P}$  accumulation, it is not necessarily accurate to evaluate accumulation or biological hazard of ionising radiations to the marine environment by using information gathered from freshwater systems, and vice versa.

## Acknowledgments

We thank Mr Nick Crocker, Dr Alex Taylor and Profs William Blake, Geoff Millward (University of Plymouth) for their technical assistance and advice during experimentation. We also thank technical staff at University of Portsmouth. This work was jointly funded by the Natural Environment Research Council (NERC), the Environment Agency (EA), Radioactive Waste Management Limited (RWM) and Science and Technology Facilities Council under the Radioactivity and the Environment (RATE) programme (Grant no.: NE/L000393/1).

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## Figure legends and table captions

### Figure Legends

Figure 1. Comparative external features and anatomy of *Mytilus galloprovincialis* (left) and *Dreissena polymorpha* (right).

Figure 2. Tissue specific accumulation of  $^{32}\text{P}$  in *M.galloprovincialis* (MG, left) and *D.polymorpha* (DP, right), total activity per gram of mussel tissue in control and irradiated treatment groups. Asterisks (\*, \*\* or \*\*\*) are indicative of significant differences ( $p < 0.05, 0.01, 0.001$ ) from the corresponding control. Lower case letters denote variation between similar tissues (species specific, i.e. there is a significant difference in  $^{32}\text{P}$  bioconcentration in digestive gland between each treatment group). Upper case letters denote significant variation in similar tissue and treatment group between species (e.g. MG digestive gland tissue values [0.1, 1 and 10 mGy/d treatments] are significantly different than DP values in the corresponding treatment, there is no difference in control samples). SD is standard deviation of mean data. IMW: Internal mussel water.

Figure 3. Proportion of  $^{32}\text{P}$  in tissue after 10 day exposure in *M.galloprovincialis* (left) and *D.polymorpha* (right). IMW: Internal mussel water.

Figure 4. Activity levels ( $\text{Bq g}^{-1}$ ) in *M.galloprovincialis* and *D.polymorpha* faecal matter (dry weight), following  $^{32}\text{P}$  exposure. Asterisks (\*, \*\* or \*\*\*) are indicative of significant differences ( $p < 0.05, 0.01, 0.001$ ) from the corresponding control. SD is standard deviation of mean data.

### Table Captions

Table 1. Table illustrating custom organism option in the ERICA tool; *D.polymorpha* digestive gland (DG) and *M.galloprovincialis* DG, occupancy factors and organism geometry. Ksib and Chi are scaling parameters, representing the lengths of the minor axes in terms of length of the major axis of the ellipsoid.

Table 2. Activity levels in water samples ( $\text{Bq L}^{-1}$ ) per treatment in *M.galloprovincialis* and *D.polymorpha* (SD is standard deviation of mean data). Asterisks (\*) denote nominal.

Table 3. Order of  $^{32}\text{P}$  accumulation in soft tissue, shell and IMW in *M.galloprovincialis* and *D.polymorpha* individuals, order shows tissue with the highest to lowest bioconcentration ( $\text{Bq g}^{-1}$ ) in all treatment groups.

Table 4. Table to show (a) the expected dose rates in  $\text{mGy d}^{-1}$  and  $\mu\text{Gy d}^{-1}$  (for the ERICA tool), (b) the water activity concentrations that give the correct dose rate ( $\text{Bq L}^{-1}$ ) for both species \*as calculated from preliminary experiments, (c) the average dose rate

achieved in  $\text{mGy d}^{-1}$  and (d) ERICA tool water activity concentrations that give the correct dose ( $\text{Bq L}^{-1}$ ), corrected to 35 g whole mussel weight beaker<sup>-1</sup>.

Table 5. Table to show the expected and achieved dose rates ( $\text{mGy d}^{-1}$ ) in *M. galloprovincialis* and *D. polymorpha* digestive gland using custom geometry in the ERICA tool (Tier 2).

**Table 1.** Table illustrating custom organism option in the ERICA tool; *D. polymorpha* digestive gland (DG) and *M. galloprovincialis* DG, occupancy factors and organism geometry. Ksib and Chi are scaling parameters, representing the lengths of the

Parameter	Marine	Freshwater
	<i>M.galloprovincialis</i> : DG	<i>D.polymorpha</i> : DG
Mass (kg)	0.000176527	9.64833E-05
Height (m)	0.006	0.003
Width (m)	0.007	0.003
Length (m)	0.01	0.004
Occupancy		
Water-surface	0	0
Water	1	1
Sediment-surface	0	0
Sediment	0	0
Ksib	0.6	0.75
Chib	0.7	0.75

minor axes in terms of length of the major axis of the ellipsoid.

**Table 2.** Activity levels in water samples (Bq L<sup>-1</sup>) per treatment in *M. galloprovincialis* and *D. polymorpha* (SD is standard deviation of mean data). Asterisks (\*) denote nominal.

	Control	0.1 mGy d <sup>-1</sup>	1 mGy d <sup>-1</sup>	10 mGy d <sup>-1</sup>
<b>MG*</b>	0	709	7090	70900
<b>MG</b>	0.1 ± 0.0	535.3 ± 105.6	6911.4 ± 1101.4	70252.8 ± 5617.1
<b>DP*</b>	0	571	5710	57100

**Table 3.** Order of  $^{32}\text{P}$  accumulation in soft tissue, shell and IMW in *M. galloprovincialis* and *D. polymorpha* individuals, order shows tissue with the highest to lowest bioconcentration ( $\text{Bq g}^{-1}$ ) in all treatment groups.

Treatment ( $\text{mGy d}^{-1}$ )	<i>M. galloprovincialis</i>				<i>D. polymorpha</i>			
	Control	0.1	1	10	Control	0.1	1	10
Highest ( $\text{Bq g}^{-1}$ )	DG	DG	DG	DG	AM	DG	DG	DG
	Mantle	Gill	Other	Gill	Mantle	Shell	Other	Other
	Gill	Mantle	Gill	Other	DG	Gill	AM	Gill
	AM	Other	Mantle	Mantle	Gill	Other	Mantle	Mantle
	Other	AM	AM	AM	Other	Mantle	Shell	AM
	Shell	Shell	Shell	Shell	Shell	AM	Gill	Shell
Lowest	IMW	IMW	IMW	IMW	IMW	IMW	IMW	IMW

**Table 4.** Table to show (a) the expected dose rates in  $\text{mGy d}^{-1}$  and  $\mu\text{Gy d}^{-1}$  (for the ERICA tool), (b) the water activity concentrations that give the correct dose rate ( $\text{Bq L}^{-1}$ ) for both species \*as calculated from preliminary experiments, (c) the average dose rate achieved in  $\text{mGy d}^{-1}$  and (d) ERICA tool water activity concentrations that give the correct dose ( $\text{Bq L}^{-1}$ ), corrected to 35 g whole mussel weight/beaker.

	Expected dose rate		ERICA tool water concentrations that give correct dose rate ( $\text{Bq L}^{-1}$ )*	Average Dose rate $\text{mGy d}^{-1}$	ERICA tool water concentrations that give correct dose rate ( $\text{Bq L}^{-1}$ ) - 35 g
	$\text{mGy d}^{-1}$	$\mu\text{Gy d}^{-1}$ (ERICA)			
MG	0.1	4.17	709	0.07	993
	1	41.7	7090	0.68	9930
	10	417	70900	7.25	99300
DP	0.1	4.17	571	0.02	2250
	1	41.7	5710	0.24	22500
	10	417	57100	2.62	225000

**Table 5.** Table to show the expected and achieved dose rates (mGy d<sup>-1</sup>) in *M. galloprovincialis* and *D. polymorpha* digestive gland using custom geometry in the ERICA tool (Tier 2).

	Expected dose rate mGy d <sup>-1</sup>	Av. Achieved dose rate mGy d <sup>-1</sup>
<i>M.galloprovincialis</i>	0.1	20.76
	1	35.28
	10	468
<i>D.polymorpha</i>	0.1	0.07
	1	1.16
	10	9.22

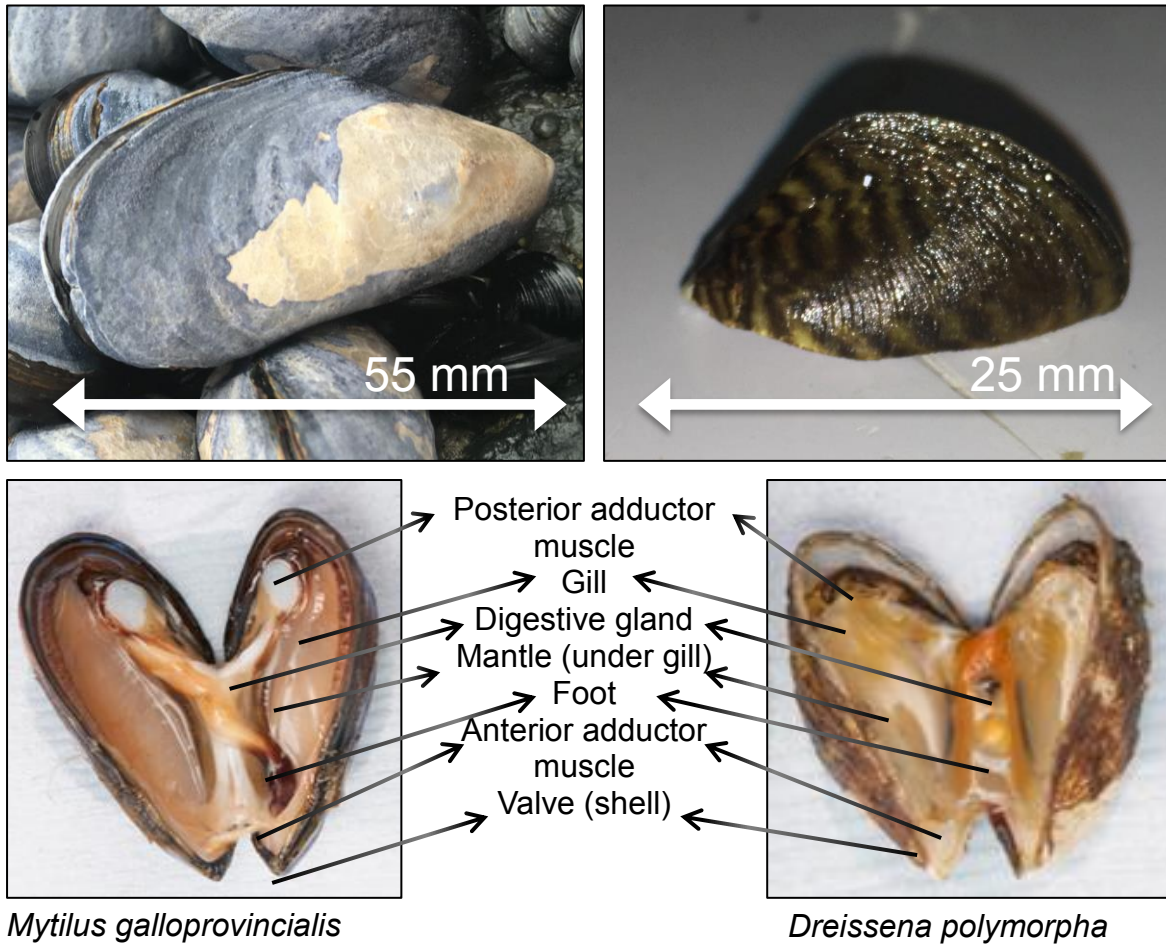


Figure 1. Comparative external features and anatomy of *Mytilus galloprovincialis* (left) and *Dreissena polymorpha* (right).

Figure 2;

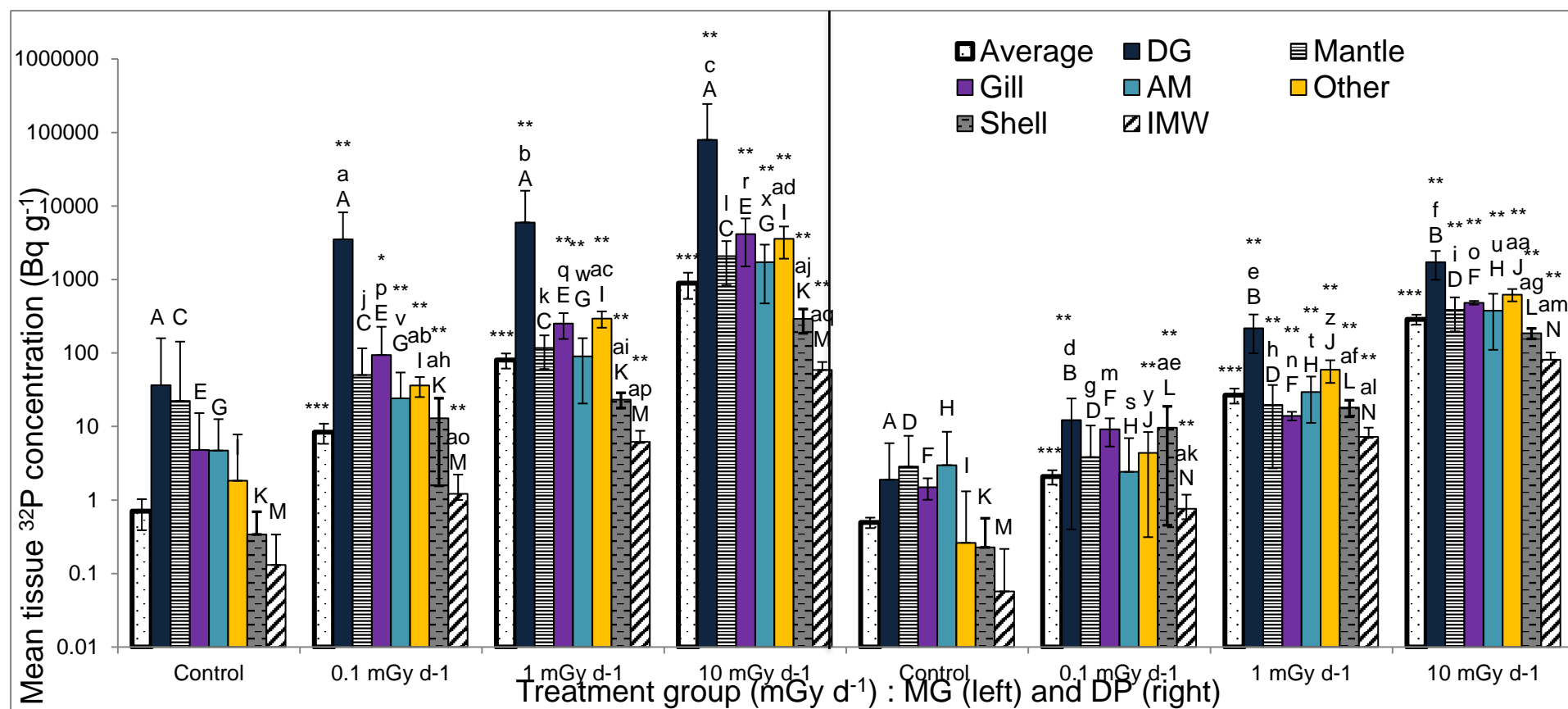




Figure 3.

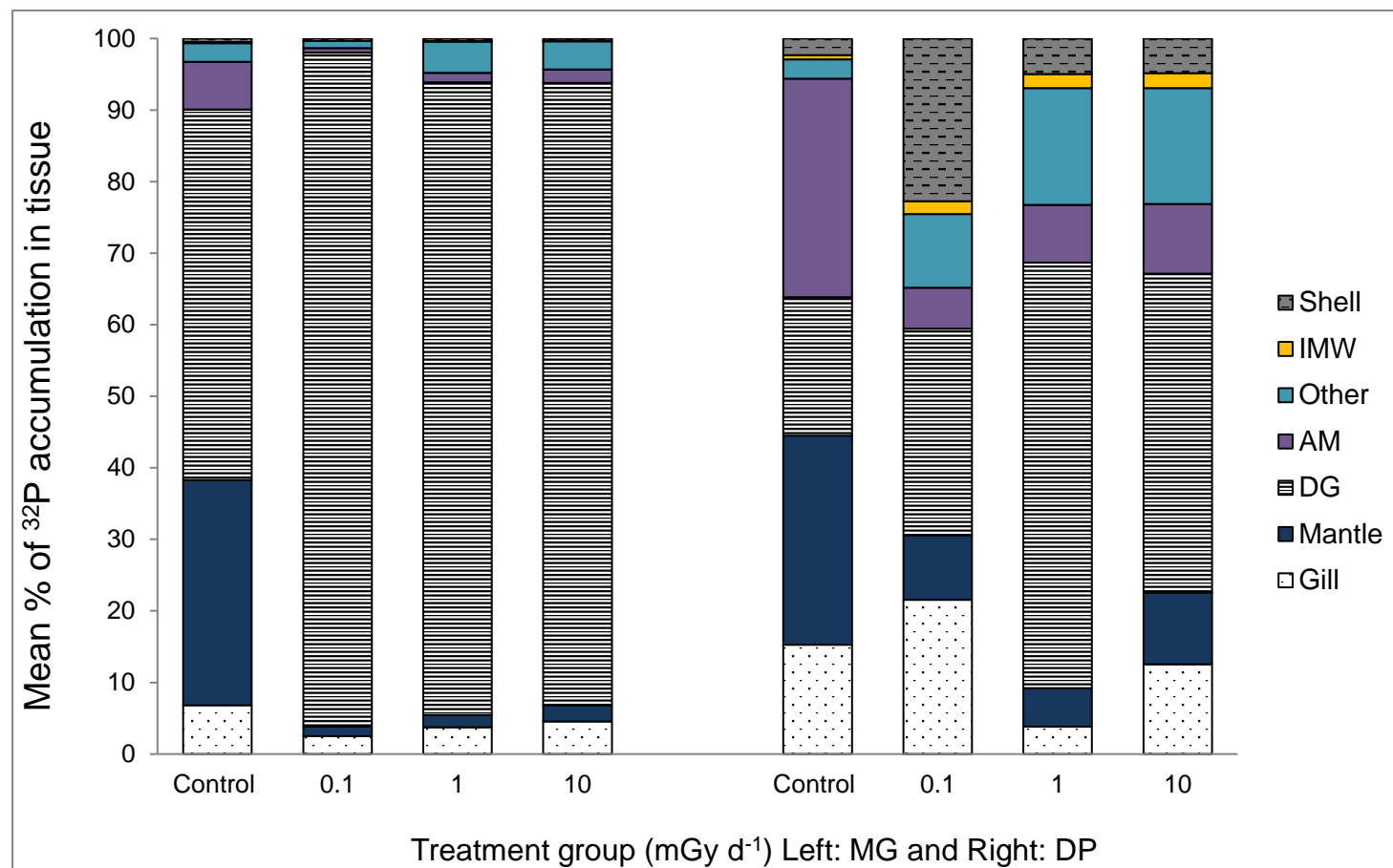


Figure 4.

